

Evaluation of N-Nitrosamine Formation in Routine Potato Cooking

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Background: Nitrosamine is amongst carcinogen chemical compounds, which can enter the human body through consumption of food. Potatoes are a root vegetable consumed by many people around the world, however their potential for nitrosamine formation during cooking processes needs to be considered for public health matters.

Objectives: In this study we evaluated the effect of conventional potato cooking method on N-nitrosamine compound formation.

Materials and Methods: The amounts of four nitrosamines, namely N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopiperidine (NPIP) and N-nitrosopyrrolidine (NPYR) were determined in four different potato-baking methods. Sixty potato samples were randomly collected from Hamadan city. Fried potato samples were roasted at 180°C and boiled potato samples were scalded at 120°C. Nitrosamine levels were measured using gas chromatography coupled with electronic ionization detector (GC-EID), and spectrophotometry was used for measuring nitrite.

Results: Fried samples that were measured by the gas chromatography method had the highest average levels of nitrosamine compounds; NDMA, 5.09 ng kg⁻¹, and NDEA, 8.66 ng kg⁻¹. Low levels of nitrosamine compounds were associated with raw potatoes, in which no nitrosamine compound was detected. Based on the analysis of the potato samples by spectrophotometry, the highest levels of nitrite was found in raw potatoes with a mean of 2.43 mg kg⁻¹ and the lowest levels of nitrite were detected in boiled potatoes with an average of 1.172 mg kg⁻¹.

Conclusions: Nitrosamine was formed with conventional potato baking methods with the most nitrosamine formation found on the surface fried samples. Nitrites amount in baked potatoes decreased. Generally, the amount of nitrosamine in baked potato samples was lower than acceptable limits.

Keywords: Nitrosamines; Nitrites; Gas Chromatography

1. Background

N-nitrosamines (NA) are small molecules with a molecular structure as follows R₂-N-NO. They form through combination of nitrate (NO₃⁻) and nitrite (NO₂⁻) with amine compounds in meat, drugs (particularly tetracycline) (1-3), detergents, and cosmetics (secondary amines, tertiary, quaternary ammonium and sometimes urea, carbamate and guanine) (4). Nitrosamines can also be formed endogenously within the human body through reaction of nitrite and amine compounds. Carcinogenic effects of nitrosamine and its ability to cause tumors in different organs such as the liver, lungs, kidneys, pancreas, larynx, and tongue has been proven by previous studies (5-8), however no carcinogenic effect for this compound has been observed in skin and bone marrow. In addition to the carcinogenic effects of nitrosamine, mutagenicity, teratogenicity and its ability to cross the placenta has also been observed (9). The first adverse effect of nitrosamine was observed in Norway during 1960 following the death

of sheep fed with fish meal containing sodium nitrite (as a preservative) because of liver toxicity (10). The toxic effects of nitrosamine compounds were discovered for the first time by Freund during 1937. Hypotheses about the carcinogenic effects of nitrosamine compounds were introduced for the first time in 1965. N-nitrosamine compounds are present in a wide range of foods in which nitrate is added for preservative effects, for example cheese, fish (because of dimethyl amine), bacon, and meat products (11). Other compounds containing nitrosamine include tobacco smoke and beer (12). N-nitrosamine compounds are also present in cosmetics such as shampoos and detergents, as well as products such as leather, plant pesticides, rubber and tires (13). One of the nitrosamines formation sources is nitrate, which enters food products in a variety of pathways, including the addition of nitrate and nitrite in food preservatives (an example is sodium nitrate that used in meat products to improve the color

and flavor and prevent the growth of *Clostridium botulinum* bacteria). Other sources of nitrate are vegetables grown using nitrate fertilizers. A large portion of nitrate consumed by humans (80% - 85%) is through vegetables (14-16). Potatoes are a root vegetable, which are part of the diet of most populations around the world. However, potatoes could contain high levels of nitrate from farmlands contaminated with chemical fertilizers (17).

2. Objectives

In this study we evaluated the effect of conventional potato cooking method on N-nitrosamine compound formation.

3. Materials and Methods

3.1. Reagents

Nitrosamine standard solution (NDELA) was purchased from Merck (Germany) and nitrite standard was purchased from Sigma (America). Sample preparation solutions, such as dichloromethane and N- (1-N-naphthyl) ethylene diamine di-hydrochloride, were obtained from Sigma, and sulfanilamide, orthophosphoric acid, ammonium acetate, potassium hexacyanoferrate, zinc acetate and sulfonyl amide chloride were purchased from Merck. Purity of all listed solutions was 99.999%.

3.2. Preparation of Samples

A minimum number of required potato samples, was estimated according to the results of a pilot study (nitrosamine was measured in nine potato samples). With an estimated mean of 0.9, standard deviation of 0.112 and a minimum difference of 15% in all categories, and with regards to the first type of error of 5% and test power of 80%, a number of 12 potato samples for each group was estimated using the Minitab Statistical package (version 14). Therefore, 60 potato samples in four groups were randomly selected. After collection, samples were washed, peeled and chopped to pieces of 1×1 cm in size. A number of 12 samples were boiled in distilled water (group 1), 12 samples were boiled in drinking water at 120°C (group 2), 12 samples were deep fried in an electric deep fryer (group 3), and 12 samples were fried superficially in a pan by sunflower oil at 180°C (group 4). The samples in each group were mixed and homogenized. The resulting samples were analyzed either directly or stored at -20°C temperature until analysis.

3.3. Preparation of Nitrosamine Mix Standard

We made a secondary storage solution with a concentration of 10 µg L⁻¹ by diluting NDELA standard solution with a concentration of 1 mgmL⁻¹. Then, by sequential dilution of the secondary storage solution in water, working standards with titrations of 1, 2, 5, 10 and 20 ngmL⁻¹ were obtained.

3.4. Extraction of N-Nitrosamines From Potatoes and Analysis by Gas Chromatography Coupled With Electronic Ionization Detector (GC-EID)

An amount of 20 g of mixed samples from each group was weighed and poured into glass vials. An amount of 20 mL of water was added to each sample and samples were shaken for 15 minutes with a mechanical shaker or one minute with rotational agitator and then centrifuged for ten minutes. Next, we transferred the sample solutions to a solid phase C18 cartridge extraction chamber that was prepared with silicagel, resine, and asbestos layers with 3 mL of methanol and 7 mL of dichloromethane and approximate flow rate of 30 mL minute⁻¹. When the nonpolar phase formed in the upper space of the cartridge we discharged the lower layer and again added methanol and dichloromethane and finally transferred the upper aqueous layer to a separator funnel and after adding dichloromethane, the sample was shaken again and the lower polar phase was discharged. If needed we filtered the solution by sodium sulfate anhydrous and then injected 0.1 µL of filtered solution into the chromatographic system.

For verification and validation of the test method, with the addition of certain concentrations of nitrosamine solution into the control solution and injection to GC and comparing the peak area with the calibration curve, the recovery rate was calculated. In this study the detection limit was 0.001 ngkg⁻¹, limit of quantitation 0.003 ng kg⁻¹, and the recovery rate was 103% (1, 12, 18-20).

3.5. Determination of the Amount of Nitrite in Potatoes With Spectrophotometry

After sample preparation using the above-mentioned method, 10 g of each sample was weighed and extracted with warm water and saturated borax, then, the proteins were precipitated with potassium hexacyanoferrate and zinc acetate, and finally the resulting precipitate was filtered out.

Adding sulfonyl amide chloride, HCL and N- (1-N-naphthyl) ethylene diamine di-hydrochloride to filtered solution resulted in a red complex, and finally the nitrite concentration was measured by spectrophotometry at 538 nm to 540 nm wavelength (21).

3.6. Statistical Analysis

The results of measurements of nitrosamine compounds by gas chromatography and nitrite by spectrophotometry were analyzed using one way Analysis of Variance (ANOVA) and Tukey's post-hoc test with the SPSS software version 22.

4. Results

The amount of N-nitrosodimethylamine (NDMA) in raw potato and potatoes cooked with different methods (deep frying, superficial frying, boiled in distilled water, and drinking water) are shown in Table 1. The NDMA level

in raw potato was significantly different from deep fried and superficially fried potatoes ($P < 0.05$), yet there was no significant difference between NDMA levels of raw and boiled (distilled water and drinking water) potato samples. The amount of NDMA in deep fried potatoes was significantly different from the NDMA amount in superficially fried potatoes yet didn't differ from potatoes boiled in distilled and drinking water. The NDMA levels in superficially fried potatoes were significantly different from boiled potatoes in distilled water and drinking water. The NDMA levels of potatoes boiled in distilled water did not differ from potatoes boiled in drinking water. Table 2 shows the NDEA (N-nitrosodiethylamine) of raw potato samples and potatoes cooked by deep-frying, superficial frying, boiling in distilled water, and drinking water. Using the ANOVA test followed by Tukey's test, it was shown that the amount of NDEA in raw potato samples was significantly different from the amount of NDEA in potatoes cooked by deep frying and superficial frying, however, there was no significant difference between the amount of NDEA in raw and boiled potatoes (boiled in distilled and drinking water). The NDEA levels in deep-fried and

superficially fried potatoes were similar yet were different from potatoes boiled in distilled water and drinking water. The NDEA in superficially fried potatoes was significantly different from potatoes boiled in distilled and drinking water. There was no significant difference between the amounts of NDEA in potatoes boiled in drinking water and distilled water. No N-nitrosopiperidine (NPIP) and N-nitrosopyrrolidine (NPYR) were found in any of the potato samples. Nitrite levels in raw potato samples and potatoes cooked using the four cooking methods are shown in Table 3. The level of nitrite in raw potato samples was not different from potato samples cooked by deep-frying and superficial frying methods, however it was different from potato samples boiled in distilled and drinking water. Nitrite levels of deep-fried and superficially fried potatoes did not differ, however they were significantly different from samples boiled in distilled and drinking water. The level of nitrite in superficially fried samples was significantly different from samples boiled in distilled and drinking water. Nitrite level of samples boiled in distilled water was significantly different from samples boiled in drinking water.

Table 1. Comparison of the Level of N-Nitrosodimethylamine in Raw Potato Samples, and Potatoes Cooked Using Deep Frying, Superficial Frying, Boiling in Distilled, and Drinking Water

Cooking Method	Values ^a	Statistical Tests
Raw	0 ± 0	F = 15.75
Deep frying	2.235 ± 1.3964	
Superficial frying	5.092 ± 3.514	df = (4, 55)
Boiling in distilled water	0.619 ± 0.797	
Boiling in drinking water	1.183 ± 0.577	P < 0.001

^a Data are presented as mean ± SD and based on mg kg⁻¹.

Table 2. Comparison of the Level of N-Nitrosodiethylamine in Raw Potato Samples and Potatoes Cooked Using Deep Frying, Superficial Frying, Boiling in Distilled, and Drinking Water

Cooking Method	Values ^a	Statistical Tests
Raw	0 ± 0	F = 20.78
Deep frying	5.78 ± 3.31	
Superficial frying	8.66 ± 4.59	df = (4, 55)
Boiling in distilled water	1.29 ± 1.65	
Boiling in drinking water	1.69 ± 1.74	P < 0.001

^a Data are presented as mean ± SD and based on mg kg⁻¹.

Table 3. Comparison of the Level of Nitrite in Raw Potato Samples and Potatoes Cooked Using Deep Frying, Superficial Frying, Boiling in Distilled, and Drinking Water

Cooking Methods	Values ^a	Statistical Tests
Raw	2.43 ± 0.845	F = 7.316
Deep frying	1.88 ± 0.702	
Superficial frying	2.099 ± 0.862	Df = (4, 55)
Boiling in distilled water	1.172 ± 0.531	
Boiling in drinking water	1.204 ± 0.850	P < 0.001

^a Data are presented as mean ± SD and based on mg kg⁻¹.

5. Discussion

Nitrosamine compounds are carcinogenic chemicals that enter the human body via many pathways. Food intake is one of the most important ways of nitrosamine entrance into the human body. As previously mentioned, amine compounds, nitrates, and nitrites are precursors of nitrosamine compounds and combine to form these compounds both in food (during preparation, processing, and preservation) and in the body at a particular pH and temperature. Among the N-nitrosamine compounds, NDMA and NDEA have received special attention because of their mutagenic and carcinogenic potential in various organs such as the liver, lungs, kidneys, bladder, pancreas, larynx, and tongue (20).

The results of nitrosamine compounds measurement by gas chromatography showed that nitrite exists in raw potatoes and baking operations can decrease its levels, although conventional methods of cooking lead to the formation of nitrosamine compounds in potatoes. Therefore, applying thermal processes can lead to reactions between amines and nitrites that are present in potatoes and form nitrosamines. The highest formation of NDMA and NDEA occurred in superficially fried samples, which is possibly because of the longer frying process, uncontrollable heat and higher exposure to oxygen compared to deep frying, and loss of greater amounts of moisture in comparison to boiling methods. The lowest level of nitrosamine compounds were found in raw samples followed by boiled potatoes, which is because of nitrite's solubility in water; this ion is discharged from the potato matrix to the surrounding water.

John Alexander et al. studied the change in the amount of nitrite and nitrate of vegetables cooked by different methods in 2008. The results showed that the amounts of nitrate and nitrite decreased by 16 to 62% and 16 to 98%, respectively, when potatoes were cooked using different methods (boiling, microwave, steaming, and frying). The highest reduction of nitrate (36% - 58%) and nitrite (82% - 98%) was found for peeled potatoes boiled in water. Frying potatoes reduced nitrate by 50% - 62%. Generally, preparation and cooking methods reduce the amount of nitrate and nitrite (22). This study was in agreement with the results of our study, as our study also showed the highest reduction of nitrite with the boiling method and the lowest reduction with the frying method.

Bara V et al. in Romania analyzed samples of meat, fish, dairy products, vegetables, fruit, and soup for N-nitrosamine by the GC/MS method during 2011 (1). This compound was not detected in raw meat. In cured meat products, five nitrosamines including NDMA and NDEA were detected while NPYR was only detected in fried bacon. Nitrosamines level increased after cooking. In fish and fish products, salted and smoked fish had the highest nitrosamines level, yet this compound was not detected in cooked fish. This study showed the presence of nitrosamine in raw fish because of trimethylamine. In

most types of cheese to which nitrate was added, NDMA was detected. Nitrosamine was not detected in milk and cooked egg, despite amines. Nitrosamine has not been detected in vegetables (fresh, frozen and canned), fruit, baby food, soups, ready-to-eat foods, dough-based foods, cooked foods, salads, and milk. According to this study n-nitrosamine compounds were not detected in fresh, frozen, and canned vegetables, which is in contrary to our results. This may be because of differences between preparation, cooking condition and type of vegetables.

This study showed that N-nitrosamine compounds were formed in conventional potato cooking. In this study we investigated the effect of thermal processes and the amount of nitrosamine was identified. Various conditions can affect nitrosamines formation, for example spices such as pepper can be a source of NPYR formation (12, 22). We suggest that future studies should investigate different conditions and ingredients that can influence the amounts of nitrosamines in food products. There was a reduction of nitrite during cooking potatoes and the highest reduction of nitrite occurred with boiling methods and the lowest reduction occurred with the superficial frying method. Other vegetables need to be evaluated separately because of their different chemical and textural structure and different cooking methods. Checking content of other vegetables, such as spinach and beetroot, for nitrites and amines is recommended to identify potential nitrosamine formation in cooking processes.

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Authors' Contributions

Maryam Ahmadi and Payman Qajarbeygi, developed the original idea and the protocol, abstracted and analyzed the data, wrote the manuscript, and were the guarantors. Ashraf Haj Hoseini, Asghar Mohammad Poorasl, Razzagh Mahmoudi and Maryam Ataee contributed to the development of the protocol, abstracted the data, and prepared the manuscript.

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